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Genetic and Molecular Research of Resistance to Wilt in Cotton: A Concise Review

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A B S T R A C T

One of the key natural fiber of the world is Cotton (*Gossypium* spp.), a member of Malvaceae family. Fusarium wilt (FW) is majorly caused by the *Fusarium oxysporum* f. sp. vasinfectum (FOV) which is a soil-borne fungus that leads to Verticillium Wilt. It is one of the most serious diseases in cotton, has a deterioting influence on crop's production and quality. *Helicoverpa armigera*, known as the cotton bollworm, has been controlled over the last few years, Verticillium Wilt (VW) has become a key restriction in production of cotton. DNA molecular markers are widely used for establishing genetic and physical genome maps, differentiating individuals, investigating genetic relatedness, and studying genome organisation of a large population with accuracy. The wilt had become a matter of research in cotton-resistance hereditary qualities, rearing and plant pathology. This work is consists of a literature review that is eventually providing a complete union of research progress in plant breeding, hereditary qualities and molecular mapping of the cotton genome with molecular markers for identification of fusarium wilt. Enrolment of Fusarium wilt resistant cultivars has demonstrated to be the most economically worthy method to control the disease. It is expected that new plant breeding methods and new varieties/hybrids resistant to Verticillium wilt will be developed in no time which can combat Fusarium Wilt.

Introduction

Cotton crop outputs are the major natural fiber in the world and as well as oilseed crops (Constable and Bange, 2015). While cotton is mostly grown in 80 countries, India leads in cotton production followed by China, United States, Pakistan and Brazil (Khan *et al.*, 2020). Malvaceae family includes *Gossypium* species which comprises of 90 genera, including the genus Gossypium. Species of this genus shows development of bushes and saplings up to seven feet tall with an inclination of whole leaves rotating with

stipules. *Gossypium hirsutum* and *Gossypium barbadense* are exemplary allopolyploids that risen up out of the mix of two earlier separated diploid genomes, containing one genome identified with those found in the Old World (A-genome diploids) and a subsequent genome identified with those of the New World (D-genome diploids) (Zhao *et al.*, 1998).There are 46 diploid and 7 tetraploid cotton species after molecular affirmations and taxonomy indicated two new tetraploid ones, *viz.*, *Gossypium ekmanianum* (AD6) and *Gossypium stephensii* (AD7) (Gallagher, 2017; Grover, 2015). Among these, solitary four are cultivated all around the globe: two species of diploids (2n = 2x = 26) and two of allotetraploids (2n = 4x = 52). Global cotton production is manifested from the two allotetraploid species *Gossypium hirsutum* and *Gossypium barbadense* (Wendel, 1992, 2013; Wang, 2015).

During the growth of cotton, it is prone to diseases like Fusarium wilt (FW) and Verticillium wilt (VW) which can show severity at some stage. These diseases tend to make the harvest yellow, shrink and fall, it can likewise harm the vascular tissue and at last can cause death of the plant. So consequently, they are likewise called as the 'cancer' of cotton crops (Zhang *et al.*, 2018).

In past few years, critical epidemics of cotton Verticillium wilt have happened for various reasons, for example, the worldwide changes in the atmosphere and environment, the long-term uninterrupted cropping of cotton and the frequent introduction of new cotton varieties/hybrids from different regions (Guo *et al.*, 2015). It is one of the vital soil-borne disease with a wide scope of hosts (Sal'kova *et al.*, 1965). The variation and divergence of *Verticillium dahliae* strains have headed towards the shortage of resistant cotton cultivars. From 2009 to 2010, China (5.0-6.6 million acres) was influenced by Verticillium wilt disease in more than 50% of the cotton growing region (National Cotton Council of America - Disease Database; Zhang *et al.*, 2013).

To determine genetic diversity, DNA based markers which includes restriction fragment length polymorphisms (RFLPs) (Ashokkumar, 2011), random amplified polymorphic DNA (RAPD) (Zhang, 2005; Shen, 2007; Pang, 2009), amplified fragment length polymorphisms (AFLPs) (Rakshit, 2010), simple sequence repeat (SSR) (Freire, 2008; Song, 2009; Liang, 2013), expressed sequence tags (ESTs) (Wang, 2013), inter-simple sequence repeat (ISSR) (Cao, 2014), and single nucleotide polymorphisms (SNP) are used (Majeed *et al.*, 2019). While comparing with other biomarkers, SSR has additional benefits which include co-dominant inheritance, more reproducibility, distribution all over the genome and it is highly informative, reliable, transferable (Ditta *et al.*, 2018).

Several quantitative trait loci (QTL) linked to Verticillium wilt disease have been labelled utilizing family-based QTL mapping strategies by gathering isolating populations of two cotton cultivars having distinction in Verticillium wilt resistance (Bolek *et al.*, 2005; Yang *et al.*, 2008; Wang *et al.*, 2008; Jiang *et al.*, 2009; Ning *et al.*, 2013; Fang *et al.*, 2013; Li *et al.*, 2013; Zhang *et al.*, 2014; Zhang *et al.*, 2014) and by association mapping techniques (Zhao *et al.*, 2014).

Fusarium wilt is presently not, at this point one of the significant diseases in cotton in the United States of America (USA) since the 1980s (Blasingame and Patel, 2013) and in China since 1990s (Jian *et al.*, 2003, Shen and Chen, 1982; Tan, 1982; Ma and Jian, 1994). However, *Fusarium oxysporum* f.sp. vasinfectum (FOV) race 4 in California since the 2000s and a harmful strain in Australia

since the mid 1990s have created as one of the significant dangers to cotton production (Allen, 2007; Colyer, 2007; Kirkby *et al.*, 2013). Fusarium wilt can go under control, mostly because of human endeavors, while controlling Verticillium wither, particularly the defoliating type, has become progressively more compelling following by the management of bollworm in Bt cotton in last couple of years. Scarcity of resistant cotton plants have been caused due to the variation and differentiation of *Verticillium dahliae* strains (Wan *et al.*, 2012).

Genetic diversity in various plant species plays a fundamental part of crop production in farming, including cotton. Variability in genes in the *Gossypium* species is available all around the globe, covering enormous geographic and natural specialities. It is an imperative wellspring of conserved genetic diversity in situ in Mexico for cotton origin (Gutiérrez, 2009; Myers, 2009) and is safe guarded ex situ inside overall cotton germplasm assortments and materials of breeding programs.

Mapping of wilt resistance quantitative trait loci and related genes

A quantitative trait locus is basically a segment of DNA that associates with a variation of a quantitative trait in the phenotype of a population (Miles and Wayne, 2008). These are mapped by using molecular markers (such as SSRs and AFLPs) which correlates with an observed trait. The stability of QTL and its advance use in breeding: QTL mapping is basically for marker-assisted selection (Cobb *et al.*, 2019). In this review, major QTLS related to wilt in both field as well as protected cultivation were acknowledged. It should be noted that this review just indicates the effectiveness of QTL in both resistive and susceptible genotypes. In view of QTL mapping, Marker assisted selection uses gyrated markers on both the sides which were then joined with phenotype screening in an enormous population, recommended to improve selection proficiency for crop improvement purpose (Collard and Mackill, 2008).

Bolek *et al.*, (2005) mapped F2 population obtained from highly tolerant Pima S-7 and susceptible Acala 44 and used microsatellites to uncover polymorphism among susceptible and resistant parents. 225 SSRs marker pairs screened bulk comprised by 10 each resistant and susceptible progeny. 60 markers were utilized to break down QTLs and 11 linkage groups were built using 35 markers and spreading over to 531cM with a mean distance of 15.17 cM. They found that 15 markers have huge linkage affiliation and nine were conveyed to chromosomes 10, 11, 12 and 25. 3147-2, CM12 and STS1 loci had a major effect on verticillium wilt resistance. On chromosome 11, LG-1 had two loci while LG-2 had one.

Wang *et al.*, (2009) found fusarium wilt resistant gene using molecular mapping firmly linked to the SSR marker "JESPR304-280" on chromosome D3(c17) and named it as FWR. Four QTLs for fusarium wilt resistance were also detected viz., CHR.A79c7), D3, D9(c23) and D1(c15). One significant QTL (LOD>20) was found near marker JESPR304 within a short period of 0.06-0.2 cM and portrayed 52.5-60.9% of total phenotypic variation. The trial affirmed the nearness of a significant quality in Chr.D3 and it was the absolute first report of molecular mapping accomplished for resistance to fusarium wilt in cotton.

Ulloa *et al.*, (2011) evaluated F2 and RILs of interspecific populations of Pima-S7 and Upland TM-1 respectively and QTL mapping indicated inheritance and interaction of nine cotton chromosomes while major QTLs were

distinguished on five chromosomes viz., Fov1-C06, Fov1-C19, Fov1-C08, Fov1-C16 and (Fov1-C111 and Fov1-C112) loci and explained 8-31% phenotypic variation. Fov1-C16 loci in F2 populations and RILs assumed a key job in presenting FOV race 1 resistance.

Wilsom *et al.,* (2012) evaluated genetic base of MCU-5 which is resistant to FW and found three QTLs in F3 and eight in F4 which clarified 9-41% of phenotypic variation. These were situated on four linkage groups including A6 (Chr 6), D6 (Chr 25) and D4(Chr 22) with two QTL situated in a comparable region as found in Sea Island Cultivar Pima 3-79 which is FW resistant.

Ullo *et al.,* (2013) observed a unique resistance gene (Fov 4) template in F2 populations of *G. hirsutum* x *G. hirsutum L.*, *G. hirsutum* x *G. barbadense* and *G. barbadense* x *G. barbadense L.* based on inheritance of phenotypes. The FOV4 gene was found to be situated close to genomic region of chromosome 14 labelled as QTL FOV4-C141 and it contributed maximum to FOV race 4 resistance produced in F2 progeny. Additionally recognized a lot of 11 SSR markers which showed the contribution of quality and quality connection between six linkage chromosomes viz., 3, 6, 8, 14 and 25 in legacy of FOV race 4 resistance. QTLs with minor impacts portrayed the 5-19% variety.

Zhang *et al.,* (2014) utilized interspecific chromosome fragment introgression lines to recognize QTLs linked with verticillium wilt in cotton and vaccinated with three defoliating *V. dahliae* strains. 42 QTLs comprising of 23 with increasing resistance and 19 with diminishing affected host resistance in contrary to three strains. 18 chromosomes were identified for mapping of these QTLs viz., A1, A3, A4, A5, A7, A8, A9, A12, A13, D1, D2, D3, D4, D5, D7, D8, D11 and D,12 having LOD value from 3-9.29.

Zhang *et al.,* (2015) mapped 10 resistance QTLs in backcross inbred lines for verticillium wilt dependent on 2895 cM linkage map with 392 SSR markers. Genetic diversity of VW resistance in cotton was likewise concentrated in 4 year replicated trial and ten VW resistance QTLs were identified. Out of 306 QTLs and linked SSR markers, 28 disease resistance QTL clusters and 24 hotspots were determined and estimated information for MAS and high resolution mapping of resistant QTLs and genes.

Zhang *et al.,* (2015) detected approximately 40 QTLs on 19 chromosomes in five FW resistant sources of USA viz., Dillon, Dixie Triumph, Cook 307-6, Wild and Coker Clevewilt and three from China viz., Chuan 52-128, Chuan 57-681 and CRI 12. Zhao *et al.,* (2014) evaluated 150 elite *G. hirsutum* L. germplasm from around the world for marker-assisted selection of verticillium wilt resistance in cotton. They identified 42 QTLs with verticillium wilt resistance by association mapping which was scattered among 15 chromosomes. 10 QTLs were previously identified and 32 were new unreported. QTL clusters for VW resistance were also proved on chromosome 16 in this study.

Xiu-hua *et al.,* (2016) used 13 microsatellites markers flanking QTLs and developed 155 cotton inbred lines through pyramiding diverse QTLs identified with verticillium wilt resistance from generations obtained by crossing 5026 and 60182. They identified four superior QTLs viz., q-13?NAU6598-1, q-6/NAU2754-2, q-5/NAU905-2 and q-8/NAU3053-1 respectively.

Palanga *et al.,* (2017) reported 119 QTLs of disease incidence and disease index on 25

chromosomes of cotton genome with the exception of chromosome 13 (c13). For disease index, 62 QTLs explained 3.7-12.2% phenotypic variation were found on 24 chromosomes with the exception of c11 and c13. Seven QTLs were found stable at all situations among which six have sGK9708 allele. For disease incidence, 59 QTLs explained 2.3-21.30% of phenotypic variation were found on 19 chromosomes except c5, c8, c12-c13, c18-c19 and c26. 28 QTLs were steady in all environments. On chromosome 13, 18 QTL clusters consists of 40 QTLs were categorized *viz.*, c1-c4, c6-c7, c10, c14, c17, c20-c22 and c24-c25.

Wang *et al.*, (2018) detected qVWR-16-1a between two markers viz., BNL2986 and NAU751 on chromosome 16 with 5.73 cM and accounted for 16.53% of phenotypic variation and resistance in F2 and F2:3 populations of *G. hirsutam* x Luyuan 343 introgressed from *G. barbadense* L. qVWR-16-1b was located on the same interval towards qVWR-16-1a with a distance of 1.73 cM to locus NAU751 and explained for 10.27% phenotypic variation. qVWR-16-2b is situated on another stretch somewhere in the range of BNL1604 and BNL1395 on the same chromosome fragment with 1.39 cM to BNL1395 and accounted for 10.8% phenotypic variation. qVWR-2-1b was located somewhere in the range of BNL3950 and BNL3971 with 0.01 cM to BNL3950 on chromosome 2 and accounted for 13.78% of phenotypic variation. Subsequently, gene pyramiding the resistant genotypes of marker BNL1395 and NAU751 can enhance VW resistance.

Marker assisted selection for wilt resistance in cotton

Simple sequence repeat (SSR)

These were established by Litt and Luty (1989) and by Edwards *et al.,* (1991) firstly in humans and they were lately employed to plants by Akkaya *et al.,* (1992). They are also known as "microsatellites" and has a major potential to be utilized as a flexible device in molecular breeding because of their property and capability for predicting variation among cultivars. (Adato *et al.,* 1995; Levi and Rowland, 1997).

Zhen *et al.,* (2005) and colleagues mentioned that the distance between a locus linked to Verticillium wilt resistance and the SSR marker BNL3556 is 13.1 cm which represented for 50.1% of phenotypic variance in 175 F_2 individuals obtained from a cross of *G. barbadense* (α15-3493) × *G. hirsutum* (Shihezi875). The DNA of 10 each resistant and susceptible individuals were then enhanced & utilized to create resistant and susceptible genetic stocks which were screened with 768 pairs of SSR primers in which BNL2440 and BNL3255 both exhibited polymorphisms among resistant and susceptible DNA pools. BNL3255 primer amplified a fragment of 208bp which was named as BNL3255-208. BNL3255-208 and the verticillium wilt resistance locus has a genetic distance of 13.7cM.

Du *et al.,* (2006) determined 510 lines out of which 155 cotton introgressed lines from interspecific hybridization were detected and results predicted specific SSR locus among interspecific lines with diverse exotic gene sources and the more specific loci with exotic genes the more resistance to verticillium wilt.

Wang *et al.,* (2007) evaluated 95 upland cotton genotypes and used 19 SSR primers and the results predicted that 19 SSR primers created 89 DNA bands in which 61 were observed polymorphic. The absolute number of genes per locus ranged from 3-8 with an average of 4.7. The polymorphic information content an incentive for SSR amplification

varied from 0.978-0.998 with an average of 0.69.

Amplified fragment length polymorphism (AFLP)

These are polymerase chain reaction (PCR) based markers which are like to randomly amplified polymorphic DNA (RAPD) and restriction fragment length polymorphisms (RFLP) analysis which can be carried out on genomes of any cultivar and complexity. It is widespread and a multilocus marker and basically requires PCR amplification of restriction fragments of total double-digested genomic DNA (Katherine Reyes and Marcus Zervos, Molecular Diagnostics, 2010)

Qi *et al.,* (2000) evaluated cotton and revealed on susceptible and four resistance related DNA fragments of 200-525 bp length. Set of two primers were used to enhance DNA fragments and acquired two bands *viz.*, resistive (292 bp) and susceptive (410 bp) and the distance between them was 13.49 cM whereas distance among resistance gene marker and resistance gene was 20.947 cM.

Zhu *et al.,* (2001) studied upland cotton and applied AFLP markers for selection in verticillium wilt and found the distance amidst resistance gene and resistance gene marker was 9.29 cM.

Abd-Elaslamet *et al.,* (2002) stated that AFLP method ustilizing silver staining to illustrate the bands is successful as applied to the molecular portrayal of *Fusarium* spp. what's more, had a solid connection between AFLP gatherings and morphological characters.

Wang and Roberts (2006) evaluated interspecific crosses of Pima S-7 x Acala NemX and Pima S-7 x Acala SJ-2 and determined that significant gene (named as Fov1) with allele dosage impact affirmed resistance from *Fusarium oxysporum* f.sp. Vasinfectum race 1 in Pima S-7. Two AFLP were linked to Fov1 with a distance of 9.3 and 14.6 cM from gene. Exceptionally resistant plants of F2 and F3 of Pima S-7 x NemX specified transgressive isolation impacts of minor genes in NemX pooled with Fov1 from Pima S-7.

Wang *et al.,* (2007) studied 95 upland cotton genotypes and used 20 EcoRI-MseI AFLP markers and the results which came out was 1480 major bands were observed. And 214 polymorphic bands. 47-109 bands per primer with an average of 74 were observed. The polymorphic information content value for AFLP amplification varied from 0.01-0.24 with an average of 0.09.

Single nucleotide polymorphisms (SNPs)

These are the broadest type of genetic variation among living beings. Each SNP speaks to a variation in individual DNA block called nucleotide. These also act as biological markers and play a major role in locating genes associated with diseases. They also play a major role when they are present within a gene or a genomic region by manipulating gene function.

Cai *et al.,* (2009) composed a haplotype map of 299 cotton germplasms and generated 1297 million reads (125 bp) comprising of 324.19 Gb of cotton genomic DNA sequence using SLAF-seq method. 649625 SLAFs were estimated and used to call SNPs had a normal profundity of 5.97 overlap per individual, 884799 SNPs have minor allele frequency (MAF) of < 5% while the remaining 85630 SNPs have MAF ≥ 0.05 and the missing rate was ≤ 50%. Distance between two genomic tags was 29.2 kb and a set of 85630 SNPs covered all the 26 chromosomes. Chromosome A08 (8764 SNPs) had the largest number of SNPs followed by A06

(6295 SNPs) and the chromosome D04 (879 SNPs) has the most modest number of SNPs.

Li *et al.,* (2017) examined 299 accessions and detected 85630 SNPs using a particular locus amplified fragment sequencing technique. They distinguished a sum of 17 noteworthy SNPs at P<1.17910-5 (P=1/85630, -log10P=4.93). Haplotype structure assessment anticipated 22 candidate traits for verticillium wilt resistance based on A10_99672586 with a minimum P-value (-log10P=6.21). CG02 was close to SNP A10_99672586 (0.26 Mb) situated in a 372 kb haplotype square and its Arabidopsis AT3G25510 homologues contain TIR-NBS-LRR domain which may be engaged with disease resistance. Rt-PCR and virus-induced gene silencing (VIGS) portrayed that CG02 was explicit in resistant genotype, Zhongzhimian2 (ZZM2), therefore, it indicates that CG02 is an aspirant gene for resistance against *V. dahlia* in cotton.

Li *et al.,* (2017) used a board of 299 accessions of upland cotton and trait SNP association detected 17 major SNPs at P<1.17 x 10-5 (P=1/85630, -log10P=4.93). They predicted 22 candidate genes for verticillium wilt resistance dependent on A10_99672586 with P > -log10P = 6.21.

Random amplified polymorphic DNA (RAPD)

These are DNA segments which are enhanced by PCR with the help of short synthetic primers usually of 10 bp in size of any random or irregular sequence. These oligonucleotides have the tendency to operate as both forward and reverse primer and are additionally ready to amplify segments of 1-10 genomic sites at same time (Hadrys *et al.,* 1992).

Fang *et al.,* (2001) obtained a RAPD marker with OPB-191300 connected with wilt resistance in cotton and found genetic distance to be 12.4 cM with 12.1% variation.

Zhang *et al.,* (2002) amplified the DNA of 58 glandless cotton varieties using 15 diverse primers. 50 bands (47.20%) were polymorphic out of 106 RAPD identified bands, thus, varieties were classified into six groups.

Sequence tagged sites (STS)

These are short DNA fragmenrs of size 200 – 500 bp which have a solitary occurrence in genome whose base sequence and locus is known (Olson *et al.,* 1989).

Fang *et al.,* (2013) designed a total of 72 primers using 36 unique RGA-AFLP sequences which resulted in formation of only one polymorphic STS marker. In BILs population, 7 primers were used and the polymorphism ratio ranged between 11.1 – 82.6% with an average of 31.1& whereas in RILs, 8 primers were informative and polymorphism ratio ranged between 9.1 – 89.3% with an average of 18%.

Wilting in cotton is turning into a significant zone of research in cotton resistance genetics, molecular breeding and plant pathology. Employments of markers have added helpful data to the comprehension of the genetic base of Verticillium Wilt resistance in cotton. With the distinguish understanding of many potential DNA markers in plant breeding, researchers implemented capability using Marker Development. In plant breeding, marker assisted selection is being used as a molecular tool for the utilization of DNA markers associated with agronomical important genes and other genes linked with biotic and abiotic stress resistances by selecting phenotype using genotype markers. The utilization of the molecular marker innovation in plant breeding unwrapped new

ways for crop improvement is known as molecular breeding. This review thus helps in investigating the ongoing research exploration on molecular breeding strategies for resistance and molecular markers.

Conflicts of Interest

The authors declare no conflict of interest.

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